

Listing of Claims:

Claim 1. (Original) An assay for determining the cyclooxygenase- 2 activity of a sample comprising the steps of:

(a) adding

- (1) a human osteosarcoma cell preparation,
- (2) a sample, said sample comprising a putative cyclooxygenase-2 inhibitor, and
- (3) arachidonic acid; and

(b) determining the amount of prostaglandin E₂ produced in step (a).

Claim 2. (Original) An assay for determining the cyclooxygenase-2 activity of a sample according to claim 1 comprising the steps of:

(a) adding

- (1) a human osteosarcoma cell preparation,
- (2) a sample, said sample comprising a putative cyclooxygenase-2 inhibitor, and
- (3) arachidonic acid; and

(b) determining the amount of prostaglandin E₂ produced in step (a),

wherein the cell preparation comprises 10³ to 10⁹ whole cells of osteosarcoma per cc, or 50 to 500 ug of osteosarcoma microsomes per ml of preparation; and 0.1 to 50 µl of arachidonic acid per ml of preparation.

Claim 3. (Original) An assay for determining the cyclooxygenase-2 activity of a sample comprising the steps of:

(a) adding

- (1) a human osteosarcoma cell preparation,
- (2) a sample, said sample comprising a putative cyclooxygenase-2 inhibitor, and
- (3) arachidonic acid; and

(b) determining the amount of prostaglandin E₂ produced in step (a)

(c) correlating the amount of prostaglandin E₂ produced with cyclooxygenase-2 activity,

wherein the osteosarcoma cell preparation consists essentially of osteosarcoma 143.98.2 microsomes.

Claim 4. (Original) An assay according to claim 3 wherein the osteosarcoma 143.98.2 microsomes are substantially free of endogenous arachidonic acid.

Claim 5. (Original) An assay according to claim 3 wherein the microsomes are contacted with an amount of delipidized serum protein effective to reduce the amount of endogenous arachidonic acid in the microsomes by a factor of at least approximately 2.

Claim 6. (Original) An assay for determining the cyclooxygenase-2 activity of a sample comprising the steps of:

- (a) adding
 - (1) a human osteosarcoma cell preparation,
 - (2) a sample, said sample comprising a putative cyclooxygenase-2 inhibitor, and
 - (3) arachidonic acid; and

(b) determining the amount of prostaglandin E₂ produced in step (a),

(c) correlating the amount of prostaglandin E₂ produced with cyclooxygenase-2 activity,

wherein the human osteosarcoma cell preparation contains no recombinant vector.

Claim 7. (Original) An assay for determining the cyclooxygenase-2 activity of a sample comprising the steps of:

(a) adding

- (1) a human osteosarcoma cell preparation,
- (2) a sample, said sample comprising a putative cyclooxygenase-2 inhibitor, and
- (3) arachidonic acid; and

(b) determining the amount of prostaglandin E₂ produced in step (a)

(c) correlating the amount of prostaglandin E₂ produced with cyclooxygenase-2 activity,

wherein the osteosarcoma cell preparation consists essentially of whole cells of osteosarcoma 143.98.2.

Claim 8. (Canceled).

Claim 9. (Once amended) A composition comprising:

(a) an osteosarcoma cell preparation having 8×10^4 to 2×10^6 osteosarcoma 143.98.2 whole cells per cc of cell preparation or 100 to 400 µg of osteosarcoma 143.98.2 microsomes; and 10 to 20 µl of peroxide-free arachidonic acid per cc of cell preparation; and

(b) 0.1 to 50 µl of arachidonic acid per cc of cell preparation.

Claim 10. (Original) A composition according to claim 9 wherein the microsomes are substantially free of endogenous arachidonic acid.

Claim 11. (Original) An assay for determining the cyclooxygenase-1 activity of a sample comprising the steps of:

(a) adding

- (1) a COX-1 cell preparation,

- (2) a sample, said sample comprising a putative cyclooxygenase-1 inhibitor;
- (3) arachidonic acid; and

- (b) determining the amount of prostaglandin E₂ produced in step (a)
- (c) correlating the amount of prostaglandin E₂ produced with cyclooxygenase-2 activity.

Claim 12. (Original) An assay according to claim 11 wherein the COX-1 cell preparation consists essentially of whole cells of U-937.

Claim 13. (Original) An assay according to claim 11 wherein the COX-1 cell preparation consists essentially of U-937 microsomes.

Claim 14. (Once amended) An assay according to claim 11 for determining the cyclooxygenase-1 activity of a sample comprising the steps of:

- (a) adding
 - (1) a COX-1 cell preparation,
 - (2) a sample, said sample comprising a putative cyclooxygenase-1 inhibitor;
 - (3) arachidonic acid; and

- (b) determining the amount of prostaglandin E₂ produced in step (a),

wherein the cell preparation comprises 10^5 to 10^8 whole cells of U-937 per cc, or 1 to 10 mg of U-937 microsomes per ml of preparation; and

0.1 to 50 μ l of arachidonic acid per ml of preparation.

Claim 15. (Original) An assay according to claim 14 wherein the cell preparation comprises 8×10^8 to 1.5×10^6 whole cells of U-937 per cc, or 1 to 5 mg of U-937 microsomes per ml of preparation.

Claims 16 to 18 (Canceled)

Claim 19. (Twice Amended) A transformed host cell that expresses cyclooxygenase-2 as shown in SEQ. ID. NO: 10 comprising:

(a) a mammalian or eukaryotic expression vector; and

(b) a sequence encoding human cyclooxygenase-2 comprising bases 97 to 1909 as shown in FIG. 2 (SEQ. ID. NO: 11) or encodes protein of FIG. 1 (SEQ. ID. NO: 10).

Claims 20 to 21 (Canceled).

Claim 22. (Twice amended) Human cyclooxygenase-2 cDNA comprising the coding region which is bases 97 to 1909 of SEQ. ID. NO: 11.

Claim 23. (Twice amended) Recombinant human cyclooxygenase-2 which is shown in SEQ. ID. NO: 10.

Claim 24. (Twice amended) An isolated human cyclooxygenase-2 which is shown in SEQ. ID. NO: 10.

Claim 25. (Twice amended) Purified human cyclooxygenase-2 which is shown in SEQ. ID. NO: 10.

Claim 26. (Once amended) The transformed host cell according to claim 19 wherein the expression vector is a vaccinia or baculovirus vector.

Claim 27. (Twice amended) The transformed host cell according to claim 19 wherein the cyclooxygenase-2 is expressed in COS-7 cells.